Points To Consider For The Submission of Chemistry, Manufacturing, and Controls (CMC) Information in Oligonucleotide-Based Therapeutic Drug Applications*

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*The recommendations in this presentation merely represent the agency's current thinking on this topic, and they do not preclude alternative approaches that satisfy the requirements of the applicable statutes and regulations if scientifically justified.

Oligonucleotides - A New Class of Therapeutic Agents

- Unique chemical structure
- Unique mechanism of action (e.g., antisense, immunostimulatory, RNAi)
- Solid phase synthesis
- Generally require unusual purification procedures
- Require novel analytical techniques

CFR on CMC information for INDs and NDAs

- INDs: 21 CFR, part 312.23 (a) (7)
- NDAs: 21 CFR, part 314.50 (d) (1)

Guidances for General CMC Information for INDs

1. Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs Including Well-Characterized, Therapeutic, Biotechnology-derived Products 11/1995.

2. INDs for Phase 2 and Phase 3 Studies - Chemistry, Manufacturing, and Controls Information 5/2003.

3. INDs-Approaches to Complying with CGMP's for Phase 1 Drugs (draft 1/12/2006).

Guidances for General CMC Information for NDAs

1. Guideline for Submitting Supporting Documentation in Drug Applications for the Manufacture of Drug Substances 2/1987.
2. Guideline for Submitting Documentation for the Manufacture of and Controls for Drug Products 2/1987.
3. NDAs: Impurities in Drug Substances 2/2000.

- ICH: Q1A(R2), Q1B, Q1C, Q1D, Q1E, Q2A, Q2B, Q3A(R), Q3B(R), Q3C, Q4B Draft, Q6A, Q6B, Q7A, Q8, Q9

Guidelines and Guidances

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Internet: http://www.fda.gov/cder/guidance/index.htm

Introduction

- CMC information for synthetic oligonucleotide drug substance and drug product
  - For NDAs
  - Considered to the extent applicable when submitting an IND
- Information unique to synthetic oligonucleotide drug substance and drug product to assure
  - Identity
  - Quality
  - Purity
  - Strength
- Additional information may be required in case of double stranded oligonucleotides (e.g., siRNA), cyclic oligonucleotides, modified oligonucleotides, and oligonucleotide conjugates.

DRUG SUBSTANCE

Description & Physicochemical Characteristics:

- Nomenclature
- Molecular Formula
- Optical Rotation
- pKa
- Hygroscopicity
- Structural Formula
- Mol. Weight
- pH
- Moisture Content
- Solubility

Proof of Structure and Characterization

Molecular Weight:

Mass Spectrometry

- Electrospray Ionization (ES)
- Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)]

Sequencing:

- Enzymatic method (e.g., Sanger)
- Chemical method (e.g., modified Maxam-Gilbert)
- Enzymatic digestion followed by MS (MALDI-TOF)

Proof of Structure and Characterization contd.

Nucleobase Composition:

Enzymatic digestion followed by HPLC of nucleosides.

Enzyme-Resistant Oligonucleotides:

Enzyme-resistant oligonucleotide → Enzyme-sensitive oligonucleotide analog.

Phosphorothioate → Phosphodiester analog.

Transformation process should not alter other parts of molecule.

Proof of Structure and Characterization contd.

Melting Temperature (Tm):

Melting temperature (T_m) using complementary DNA or RNA sequence.

Chain Length :

- Capillary Gel Electrophoresis (CGE).
- Polyacrylamide Gel Electrophoresis (PAGE).
- Provide Electropherogram.

Proof of Structure and Characterization contd.

Internucleoside Linkages:

^{31}P NMR spectroscopy:

- Phosphodiester
- Phosphorothioate
- Methylphosphonate
- Any other modified phosphate

Molecular backbone composition:

- P=S/P=O Ratio by combination of SAX-HPLC and ^{31}P-NMR.
- If single method, SAX-HPLC is preferred.

Chromatographic Profile:

- Provide HPLC chromatogram of DS
- For phosphorothioates (SAX-HPLC chromatogram)

Proof of Structure and Characterization contd.

UV:

- UV absorbance spectrum.
- Provide λ_{min} and λ_{max} for acidic, basic, and aqueous solutions.
- Extinction coefficient.

Other Spectroscopic Information:

- FTIR
- ^{1}H-NMR
- multinuclear NMR (e.g., ^{1}H-NMR and ^{13}C-NMR)

Stereochemical Composition:

- State stereorandom or stereospecific synthesis
  - Both methods are acceptable.
- Number and location of new chiral centers.
- Number of possible stereoisomers (stereorandom synthesis)

Proof of Structure and Characterization of dsRNA

- dsRNA (siRNA): Overall characterization
  - combination of analytical tests
    - performed on two single strand sequences(intermediates) prior to annealing
    - Performed on annealed duplex.
- Characterization of each single strand RNA
  - molecular weight (MS)
  - molecular sequencing (MS-MS sequencing)
  - UV, and other spectral characterization.
- Characterization of double stranded RNA
  - formula weight (MS)
  - sodium counter-ion (Atomic absorption)
  - spectral characterization (include CD, Imino-proton NMR)
  - duplex content (SEC)
  - duplex melting studies (UV spectroscopic melting curve)

Starting Materials, Reagents, Solvents, and Auxiliary Materials

- List all starting materials, reagents, solvents, and auxiliary materials used in manufacturing and purification of DS. Include sources and vendor information.
- In general protected nucleoside phosphoramidites, protected nucleoside linked solid supports, and reagents such as Beaucage reagent are considered as starting materials.
- If non-commercial modified nucleosides are used, provide a description for their synthesis, proof of structure and characterization, and impurity profile information.
- Non-compendial: Provide acceptance specifications and testing procedures

Starting Materials, Reagents, Solvents, and Auxiliary Materials contd.

- Solid Phase Support: Description
  - composition
  - particle size
  - pore size
  - chemical description of linker, spacer, and loaded nucleoside and its concentration
  - dimethoxytrityl (DMT) loading (if applicable)
  - molar substitution factors
  - swelling factors (for resins)
  - stability under identified conditions (e.g., hydrolysis).

Manufacturer(s)

- Name and Address of each site
- Include all testing sites
- List responsibilities of each site

Manufacturing and Purification

Manufacturing:

Flow Diagram: Include each step of manufacturing process.

Description: Include model number and name and address of manufacturer of commercial solid phase synthesizer. Provide a detailed description for custom designed and modified synthesizers.

Manufacturing and Purification contd.

A typical solid phase synthesis cycle should include:

- Parameters for loading of solid phase support into reaction vessel/column.
- Steps involved in coupling cycle (e.g., detritylation, activation and condensation, oxidation or oxidative sulfurization, and capping).
- Cleavage of crude oligonucleotide from solid phase support.
- Pre-purification steps (e.g., deprotection of phosphate and base protecting groups).

Manufacturing and Purification contd.

Purification:

Flow diagram: Include all steps.

Provide detailed description for purification process.

Chromatography: Sample load, mobile phase, column material (including particle size and dimensions), flow rate, run time, gradient program, column temperature, detection wavelength. Criteria for collecting fractions and disposition of side fractions.

Chromatogram of representative purification run.

Manufacturing and Purification contd.

- Other Procedures:

  Deprotection of the 5'-end protecting group, for e.g. dimethoxytrityl.

  Counter ion exchange, for e.g., replacement of ammonium ions by sodium ions.

  Desalting, Depyrogenation, and Lyophilization

- For siRNA: Provide manufacturing of and description for each single strand. This should include solid phase synthesis, cleavage and deprotection, purification, and desalting and duplex formation (annealing).

In-process Controls

All appropriate in-process controls should be described

1) Verify synthesis parameters prior to initiation of actual synthesis run and provide computer print out containing synthesis parameters, if applicable.
2) Identify required reagents (e.g., phosphoramidite solutions or other synthesis reagents) used during synthesis by chemical testing or by visual reading the labels and verification by trained personnel. Proper position of bottles should also be verified.
3) If possible, monitor coupling efficiency following each extension cycle [e.g., dimethoxytrityl (DMT) cation assay of effluent obtained after deblocking step].

In-process Controls contd.

4) A minimum coupling efficiency limit should be set to ensure consistent performance of oligonucleotide synthesis.

5) After cleavage of oligonucleotide from solid support and deprotection steps, efficiency of synthesis should be assessed by analyzing crude oligonucleoitde by methods such as CGE or HPLC.

6) Completion of terminal detritylation step should be confirmed by CGE or HPLC.

In-process Controls contd.

7) After purification and desalting step, determine oligonucleotide content by UV/HPLC or CGE. For phosphorothioates, determine phosphodiesters content by SAX-HPLC.

8) If possible, determine estimated stepwise yields and, the overall yield based on DMT cation assay.

9) For siRNA: In-process controls should be provided for each single strand synthesis.

Specifications and Test Methods

Identity: Assure through a combination of two or more methods, e.g.,

- Chromatographic retention time (UV/HPLC; SEC for siRNA)
- Sequencing (MALDI-TOF, Sanger, or Maxam-Gilbert)
- Molecular weight (mass spectrometry)
- Gel electrophoretic mobility (PAGE or CGE)

In addition, include the following identity methods:

- Melting (dissociation or thermal denaturation) temperature (Tm)
- Counter ion (ICP-MS)
- Specific optical rotation (if stereochemistry is controlled)

Specifications and Test Methods

Assay: A combination of methods e.g., UV/HPLC; UV/SEC for siRNA. Report on anhydrous, solvent free, and salt free basis.

Purity:

- Deletion and Addition Sequences:
  Report, preferably by electrophoresis (CGE) or HPLC
  Individual deletion sequences (e.g., n-1, n-2 , n-3 etc.)
  Individual addition sequences (e.g., n+1, n+2 etc.)
- Phosphodiester Analogs:
  Report, preferably by SAX-HPLC
  Mono-, di-, and tri-phosphodiester analogs etc.
- Other Impurities: Depurinated sequences, aggregated sequences, and partially deprotected sequences, etc
- Individual Unidentified Impurities
- Total Impurities: Report total impurities content

Specifications and Test Methods

Organic Volatile Impurities (OVI):

Identified, unidentified, and total OVI.

Other Process-Related Impurities:

DS should be checked for presence of starting materials, intermediates, residual salts, ligands, reagents, reagent degradants (e.g., 3'-H-phosphonates), and reaction by-products such as depurinated sequences, dimethoxytrityl alcohol, benzoic acid, benzamide, isobutyramide, etc. These impurity levels should be controlled, and, if appropriate, acceptance criteria should be established.

Specifications and Test Methods

Backbone Composition:

Determine P=S/P=O ratio for mixed backbone oligonucleotides.

SAX-HPLC preferred for phosphorothioates.

Moisture Content:

Karl Fischer or alternative methods such as CGE.

pH:

Determine pH of oligonucleotide in aqueous solution.

Specifications and Test Methods

Heavy Metals: USP or ICP-MS

Microbial Controls: Depends on

- route of administration of DP
- process and controls used during manufacturing of DP
- conditions of storage

Endotoxins: If DP has endotoxin limits, control of endotoxins in DS is recommended.

Counter Ion Content: The counter ion content should be determined by such methods as ICP-MS., if applicable.

Synthesizer Validation

Prior to phase 3 studies:

- Perform instrument validation of oligonucleotide synthesizer, including computerized monomer and reagent delivery system, through:
  - Installation Qualification (IQ)
  - Operation Qualification (OQ)
  - Performance Qualification (PQ)
- Perform monomer delivery sequence validation of computerized system.
- Validation data need not be submitted in the application.

Batch Analysis

- Batch Analysis:
  - Safety batches (pharmacology and/or toxicology)
  - Clinical batches for safety and efficacy
  - Bioavailability/bioequivalence batches
  - Stability study batches
  - Representative commercial batches
    - To demonstrate consistency of manufacturing
    - To support batch release specifications.

Reference Standard

- Establish a well characterized RS.
- Prepare in a manner similar to that of drug substance.
- At a minimum should meet DS lot release specification.
- Some additional tests (e.g., nucleobase composition, spectral data, bioassay etc.) may be included.
- For IND, if RS is not established, designate a working standard as a benchmark.

Impurities

- Provide a summary of actual and potential impurities that could arise during manufacture, purification, and storage of DS. A tabular format with structures preferred.
- Isolate and identify as many impurities/degradation products as possible.
- Quantitate and qualify major impurities. ICH guidelines are not applicable, however, principles in those guidelines should be considered.

Impurities contd.

Oligonucleotide Related:

Addition sequences (n+1, n+2, etc.)

Deletion sequences (n-1, n+2, etc.)

Phosphodiester analogs

Depurinated sequences

Partially deprotected sequences

Aggregated sequences

Impurities contd.

Nonoligonucleotide-related:

- Organic molecules (starting materials, by-products, intermediates, reagents, ligands)
- Inorganic molecules (metals, inorganic salts, catalysts, cleavage reagents)
- Residual solvents
- Organic molecules (non-oligonucleotide fragments) resulting from degradation.
- Acceptance criteria should be proposed
  - Recommendations should take into consideration intended dose, duration of treatment, and route of administration.

Stability Studies

- For general stability information refer to ICH stability guidelines. Even though they are not applicable to oligonucleotides, principles in those documents may be applied.
- Conduct stability studies at intended storage and accelerated conditions.
- Demonstrate integrity of drug substance throughout clinical studies.
- Monitor physicochemical characteristics which may change over time.
- Provide statistical analysis, if performed.
- A stability indicating assay demonstrated to be capable of assessing degradation of the oligonucleotide, including deletion sequences (e.g., n-1, n-2, n-3, etc.) should be provided.

Stability Studies contd.

- For a phosphorothioated oligonucleotide, limits for the individual unsulfurized phosphodiesters (e.g., mono-, di-, tri-phosphodiesters etc.) should be provided.
- A combination of methods such as UV/HPLC may be used to assure the assay/strength and purity of the oligonucleotide.
- For Phase 1 studies, it is not necessary to provide detailed stability data, but sponsor should demonstrate that the oligonucleotide remains stable over the course of proposed clinical study.

Stability Studies contd.

- Typical stability study design should include:
  - Appearance
  - Assay
  - Degradation products (individual identified, individual unidentified, and total)
  - pH
  - Water content
  - Phosphodiester analogs (for phosphorothioates)
- Conduct one time stress studies: Acid, base, heat, light, humidity, oxidation, and etc.

DRUG PRODUCT

- Provide all applicable requirements in FDA guideline: Guideline for Submitting Documentation for the Manufacture of and Controls for Drug Products 2/1987.
- Typical Release Specifications should include:
  - Appearance
  - Identity of active ingredient (UV/HPLC, CGE, or MS; SEC for siRNA)
  - Assay for strength/potency (UV/HPLC or CGE; UV/SEC for siRNA)
  - pH
  - Fill Volume (for solutions)

DRUG PRODUCT-SPECIFICATION

Typical Release Specifications (contd.)

- Impurities/Degradation products:
  - UV/HPLC or CGE; SEC for siRNA
  - Individual identified impurity/degradation products
    - (e.g., deletion sequences n-1, n-2, n-3 etc.)
  - Depurinated sequences
  - Individual unidentified impurity/degradation products
  - Total impurities/degradation products
  - For Phosphorothioates: Individual unsulfurized phosphodiesters
    - (e.g., mono-, di-, tri- phosphodiesters etc.)
- For parenteral products:
  - Osmolality
  - particulate matter
  - sterility, and bacterial endotoxins/pyrogens tests.
- Uniformity of Dosage Units (e.g., powders requiring reconstitution) .
- Any other attributes that are specific to intended dosage form.

DRUG PRODUCT

- Container/Closure: Refer to FDA guidance document: Container Closure Systems for Packaging of Human Drugs and Biologics – Chemistry, Manufacturing, and Controls Documentation (5/99).
- Stability Studies:
  - Principles in ICH stability guidelines may be applied to oligonucleoitdes
  - For Phase 1 studies: Detailed data need not be provided. Information sufficient to assure product's stability to support the planned clinical studies.

DRUG PRODUCT- STABILITY

- Typical stability protocol should include:
  - Appearance
  - pH
  - Assay
  - Degradation products:
    - Individual identified degradation products
    - (e.g., deletion sequences such as n-1, n-2, etc.)
    - depurinated sequences
  - Individual phosphodiester anaologs (for phosphorothioate oligonucleotides)
  - Individual unidentified degradation products
  - Total degradation products
- For parenteral products:
  - Osmolality
  - Particulate matter
  - Sterility.
    - In lieu of sterility test, include container/closure integrity test annually and at expiry.
- For siRNA:
  - Strength (UV/SEC)
  - Duplex content and single strand impurities (SEC)
  - Single strand contents (SAX-HPLC), Tm etc.

SUMMARY

- Physicochemical characteristics and proof of structure
  - Include oligonucleotide specific information such as molecular weight by MS, sequencing, base composition, Tm, backbone composition, chain length, etc.
  - Demonstrate characterization of dsRNA by performing a combination of tests on single stranded sequences as well as double stranded RNA.
- Provide adequate controls for starting materials.
  - Include method of preparation and specifications for non-commercial starting materials, e.g., modified nucleoside phosphoramidites.
- Isolate and identify as many impurities/degradation products as possible in oligonucleotide DS and DP. As the drug development progresses, make efforts to increase the purity as much as possible.

SUMMARY contd.

- In DS release specifications include specific tests:
  - Identity by UV/HPLC, molecular weight by MS, Tm, counter-ion, etc. and assay and purity by UV/HPLC.
  - Report impurities such as deletion sequences, addition sequences, phosphodiester analogs (for phosphorothiotes), depurinated sequences, partially deprotected sequences, aggregated sequences etc.
  - Calculate assay on anhydrous, solvent free, and salt free basis.
  - For siRNA, include specifications for single stranded sequences as well as double stranded RNA drug substance.

- In stability protocol include:
  - Assay and impurities determination by UV/HPLC,
  - Contents of impurities/degradation products such as deletion sequences, addition sequences, phosphodiester analogs (for phosphorothioates), depurinated sequences etc.

SUMMARY contd.

- This presentation provided an overview of various CMC regulatory issues to be addressed during the course of oligonucleotide drug development process. These were only recommendations. Alternative approaches and test methods can be considered.

- A drug application should include adequate specific CMC information for oligonucleotide-based therapeutics, especially, regarding physicochemical characteristics, proof of structure and characterization, starting materials, specifications and test methods, impurity profile, batch analysis, and stability studies, so that the FDA reviewers could assess the identity, quality, purity, and strength of the oligonucleotide and its safe use in humans.

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